



**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2015

Prevalence of BRCA-1 associated protein 1 germline mutation in sporadic malignant pleural mesothelioma cases

Rusch, Andreas ; Ziltener, Gabriela ; Nackaerts, Kristiaan ; Weder, Walter ; Stahel, Rolf A ; Felley-Bosco, Emanuela

Abstract: **OBJECTIVE:** 23% of mesothelioma tumor specimens have a mutation in the BRCA1-associated protein 1 (BAP1) gene and germline BAP1 mutations predispose to malignant pleural mesothelioma (MPM). Our aim was to investigate germline BAP1 mutations in sporadic MPM patients. **MATERIALS AND METHODS:** Exonic DNA from peripheral blood leucocytes of 78 MPM patients was screened for germline BAP1 mutation. **RESULTS:** One out of 78 patients showed a germline synonymous mutation in exon 11. In all other patients wild-type sequence without any single-nucleotide polymorphisms was detected. **CONCLUSIONS:** Taking into account previous similar screenings, the prevalence of germline BAP1 mutations in sporadic MPM patients can be estimated around 1-2%, suggesting a minor role of germline BAP1 mutation in the pathogenesis of sporadic MPM.

DOI: <https://doi.org/10.1016/j.lungcan.2014.10.017>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-101876>

Journal Article

Accepted Version



The following work is licensed under a Creative Commons: Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.

Originally published at:

Rusch, Andreas; Ziltener, Gabriela; Nackaerts, Kristiaan; Weder, Walter; Stahel, Rolf A; Felley-Bosco, Emanuela (2015). Prevalence of BRCA-1 associated protein 1 germline mutation in sporadic malignant pleural mesothelioma cases. *Lung Cancer*, 87(1):77-79.

DOI: <https://doi.org/10.1016/j.lungcan.2014.10.017>

**Prevalence of BRCA-1 associated protein 1 germline
mutation in sporadic malignant pleural mesothelioma
cases**

Andreas Rusch¹, Gabriela Ziltener¹, Kristiaan Nackaerts², Walter
Weder³, Rolf A. Stahel^{1, 4}, Emanuela Felley-Bosco^{1,*}

¹ Laboratory of Molecular Oncology, Clinic of Oncology, University
Hospital of Zürich, Haldeliweg 4, 8044 Zürich, Switzerland

² KU Leuven, University Hospitals, Department of
Pulmonology/Respiratory Oncology, Leuven, Belgium

³ Division of Thoracic Surgery, University Hospital Zürich, Zürich,
Switzerland

⁴ Trial Chair SAKK 17/04, Swiss Group for Clinical Cancer Research

*Corresponding author. Tel.: +41 44 6342878; Fax: +41 44 6342872.

E-mail address: emanuela.felley-bosco@usz.ch

Abstract

Objective: 23% of mesothelioma tumor specimens have a mutation in the BRCA1-associated protein 1 (BAP1) gene and germline BAP1 mutations predispose to malignant pleural mesothelioma (MPM). Our aim was to investigate germline BAP1 mutations in sporadic MPM patients.

Materials and Methods: Exonic DNA from peripheral blood leucocytes of 78 MPM patients was screened for germline BAP1 mutation.

Results: One out of 78 patients showed a germline synonymous mutation in exon 11. In all other patients wild-type sequence without any single-nucleotide polymorphisms was detected.

Conclusions: Taking into account previous similar screenings, the prevalence of germline BAP1 mutations in sporadic MPM patients can be estimated around 1-2%, suggesting a minor role of germline BAP1 mutation in the pathogenesis of sporadic MPM.

Keywords: BAP1, Mesothelioma, Tumor predisposition syndrome, Germline mutation, BAP1 cancer syndrome, Germline mutation screening

1. Introduction

Malignant pleural mesothelioma (MPM) is a rare cancer that originates from the pleural lining and that has a strong association to asbestos. The prognosis and the treatment options at the moment are very poor with a patient median survival time of less than 12 months after diagnosis [1]. To develop more effective MPM-specific therapeutics, much effort has been put into the investigation of cancer genes driving MPM oncogenesis. Besides the two most abundant alterations in MPM concerning cyclin-dependent kinase inhibitor 2A (CDKN2A) and neurofibromatosis 2 (NF2) genes, recently genetic alterations in the BAP1 (BRCA1-associated protein 1) gene, which is localized on chromosome 3 (3p21.1), have been identified in 23% of MPM specimens [2].

BAP1 was initially identified in lung cancer cell lines as a protein that binds to BRCA1 [3]. It is a 90 kDa nuclear-localised deubiquitinating enzyme with ubiquitin carboxyl hydrolase (UCH) activity; and it is the only member of the UCH family with two nuclear localisation signal (NLS) motifs [4]. BAP1-mediated tumour suppression requires both deubiquitinating activity and nuclear localisation of BAP1 [4]. BRCA1 does not seem to be necessary for the tumor suppressor activity of BAP1 [4] and it is not a substrate of BAP1 [5]. However, BAP1 is part of essential cell cycle regulators[6] and probably associated with regulation of transcription [7]. BAP1 binds and deubiquitinates the transcriptional regulator host cell factor 1 (HCF-1), which interacts with histone-modifying complexes [8, 9]. Together, these data

indicate a complex mode of action for BAP1 involving different cellular pathways. It is even hypothesized that BAP1 effects can vary in different cell types and/or species [10]. BAP1 was shown to fulfill criteria of a genuine tumor suppressor gene, which presumably becomes apparent after a two-step inactivation according Knudson's two-hit model [4, 9, 11]: one allele of BAP1 being inactivated via inherited mutation (or monosomy of chromosome 3 [12]); and the remaining allele being lost by somatic BAP mutation(s) leading to biallelic inactivation [9, 13].

Sporadic BAP1 mutation have been described in uveal melanoma, cutaneous melanoma and other melanocytic tumors, renal cell carcinoma and other cancers [9]. Besides the common sporadic BAP1 mutations, germline BAP1 mutations have been detected in families with a high incidence of MPM [14]. Individuals with heterozygous BAP1 germline mutations are affected by a newfound tumor predisposition syndrome characterized by very high risk of developing MPM, uveal melanoma (UV), cutaneous melanoma, atypical melanocytic benign neoplasms [15, 16] and different cancer types, such as lung adenocarcinoma, meningioma [17], and renal cell carcinoma [18] (<http://omim.org/entry/614327>).

To date only two studies [13, 14] investigated the frequency of this germline mutation in 26 and 9 sporadic MPM patients, respectively, and found a prevalence of 8% and 0%, respectively. In this study, we present the largest screening to date of germline BAP1 mutation in sporadic MPM patients.

2. Materials and Methods

2.1. Patient blood samples

78 blood samples were obtained from patients of a multicenter clinical study (SAKK 17/04, NCT00334594, unpublished) with pathologically proven diagnosis of MPM and after written informed consent. Blood samples were obtained between 2008 and 2012. Staging was based on the IMIG classification [19] selecting the lowest stage in case of ambiguous results. In SAKK17/04 trial only patients diagnosed with stage I, II or III MPM were included. The study was approved by the Ethics Committee of the University Hospital Zürich. Clinical records were reviewed to document the patient's sex, age and stage and histology of malignant pleural mesothelioma.

Peripheral whole blood was collected by needle venipuncture in serum tubes. Total DNA was purified according to the protocol of Qiagen DNeasy[®] Blood & Tissue Kit.

2.2. PCR and Analysis

PCR of different fragments was performed for unreported samples to define optimal PCR conditions. Products were confirmed by electrophoresis on a 2% agarose gel and excised. After purification according to the Macherey-Nagel NucleoSpin[®] Gel and PCR Clean-up protocol products were send for Sanger sequencing (done by Microsynth AG, Balgach, Switzerland).

Then eleven PCR fragments that encompassed the entire BAP1 coding exons and adjacent intron regions were amplified for all samples including a positive control (cell lines H2452 and H28). Primers and conditions used are listed in *Supplementary Table 1*. PCR product clean up and high-throughput sequencing was done by Microsynth AG (Balgach, Switzerland).

Sequencing results were analyzed with CLC Main Workbench (Version 6.1.1, CLCbio, Aarhus, Denmark) using NCBI Sequence: NG_031859.1 as reference.

We used variation data observed in 413 exomes from the CoLaus cohort [20], which were sequenced at the Wellcome Trust Sanger Institute (WTSI) as part of a partnership between the Institute, the CoLaus principal investigators and the Quantitative Sciences dept. of GlaxoSmithKline.

3. Results

We screened peripheral blood samples of 78 sporadic MPM patients for germline BAP1 mutation. In these 78 patients there were 72 males and 6 females (*Supplementary Table II*). The median age was 61.2 years (SD ± 6.1 years) at the date of diagnosis. The histopathology showed 69 tumors (88.5%) being epithelioid, 5 (6.4%) of sarcomatoid and 4 (5.1%) of the biphasic type. The stages at the time of diagnosis distributed as follows: 15 stage I (19.2%), 37 stage II (47.4%) and 26 stage III (33.3%). As positive control we sequenced the DNA of cell lines NCI-H2452 and NCI-H28 and confirmed the already reported missense mutation c.284C>A (p.A95D) in NCI-H2452 [2, 13] and the 23-bp deletion at intron 6/exon7 junction (c.483-19_486del) in NCI-H28 [13].

The sequencing results of germline DNA showed wild-type BAP1 sequence except for patient 76, where we found a known synonymous variant in exon 11 (rs71651686, c.1026C>T, *Fig. 1*), which does not result in a change of the encoded amino acid (p.Ser342=). This patient is still alive 24 months after diagnosis and has a tumor with epithelioid histotype (*Supplementary Table II*). He has a brother who died of leukemia. In all other samples we did not find any other alterations.

4. Discussion

The screening of 78 MPM patients revealed absence of non-synonymous germline BAP1 mutation. This observation is in contrast to the study of Testa et al.[14], where 2 individuals with non-synonymous germline BAP1 mutation out of 26 sporadic MPM patients (8%) have been observed. The two patients carrying BAP1 mutations had been previously treated for uveal melanoma (UM), while none of the remaining 24 individuals had UM in history. None of the 78 patients of our study reported UM history. However, our observations are consistent with the study of Yoshikawa et al.[13]. In that study no germline mutations were detected but the number of matching non tumoral samples available was very small (nine) to draw any definitive conclusion about the frequency of germline mutation in sporadic cancer.

Altogether, 113 MPM patients were screened until to date for germline BAP1 mutation. Collectively, the prevalence of germline BAP1 mutations in sporadic MPM patients can be considered around 1-2%.

Similar screenings have been conducted for sporadic UM, where BAP1 has a predominant role in tumor predisposition syndrome. One study revealed that 2/66 (3%) unselected UM patients harbored a germline BAP1 mutation [21]. Harbour et al. found germline mutations in the BAP1 gene in 1/57 (1.75%) of UM patients [12] and Abdel-Rahman in 1/53 (1.9%) [17]. In another study [22], the prevalence of germline BAP1 mutation was 8% (4/50) in metastatic

UM versus 0% (0/50) in non-metastatic UM. Taking into account all these results, 8/276 of sporadic UM patients had BAP1 cancer syndrome, which corresponds to a prevalence of 2.9%, similar to what is observed in MPM patients.

The prevalence of the silent mutation in the exon 11, which was detected in one patient (0.6%), is consistent with minor allele frequency observed in the Swiss normal population. Since synonymous mutations are able to cause changes in protein expression, conformation and function and are not as “silent” as supposed years ago [23], the relevance of this change for predisposition to tumor development remains to be explored. It is noteworthy that we could not find any nucleotide alterations at the position c.912, which correspond to the most frequent SNP, reported in the NCBI dbSNP database (rs201809705, c.912C>A, p.Ala304=) and again this is in line with the minor allele frequency observed in the Swiss cohort, indicating that wide variation exists, stressing the importance of control population. In previously BAP1 screening reports, SNPs were not mentioned, probably because of the supposed unimportant role of synonymous mutations. Only Aoude et al.[21] reported one synonymous variant in germline BAP1 (rs28997577, on the same exon 24 nucleotides away from the synonymous variant rs71651686) out of 66 UM patients.

5. Conclusion

Taken together, the screenings performed up to the present suggest a minor role of germline BAP1 mutation in the pathogenesis of sporadic MPM. Nevertheless, if a patient reports asbestos exposure and UM in the past, or if there is a familial predisposition syndrome, germline BAP1 testing must be considered.

6. Acknowledgements

This work was supported by the Sassella Stiftung. We thank Ubiratan Moura for his skilful help with genomic DNA extraction. We are grateful for access to exome sequence data from the CoLaus cohort, which was sequenced as part of a partnership between the Wellcome Trust Sanger Institute, the CoLaus principal investigators and the Quantitative Sciences dept. of GlaxoSmithKline. We thank the Swiss Group for Clinical Cancer Research for collecting the samples.

Conflict of interest statement

The authors declare no competing financial interests.

7. References

1. Bertino P, Carbone M, Pass H. Chemotherapy of malignant pleural mesothelioma. *Expert Opin Pharmacother* 2009;10: 99-107.
2. Bott M, Brevet M, Taylor BS, Shimizu S, Ito T, Wang L, Creaney J, Lake RA, Zakowski MF, Reva B, Sander C, Delsite R, Powell S, Zhou Q, Shen R, Olshen A, Rusch V, Ladanyi M. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. *Nat Genet* 2011;43: 668-672.
3. Jensen DE, Proctor M, Marquis ST, Gardner HP, Ha SI, Chodosh LA, Ishov AM, Tommerup N, Vissing H, Sekido Y, Minna J, Borodovsky A, Schultz DC, Wilkinson KD, Maul GG, Barlev N, Berger SL, Prendergast GC, Rauscher FJ, 3rd. BAP1: a novel ubiquitin hydrolase which binds to the BRCA1 RING finger and enhances BRCA1-mediated cell growth suppression. *Oncogene* 1998;16: 1097-1112.
4. Ventii KH, Devi NS, Friedrich KL, Chernova TA, Tighiouart M, Van Meir EG, Wilkinson KD. BRCA1-associated protein-1 is a tumor suppressor that requires deubiquitinating activity and nuclear localization. *Cancer Res* 2008;68: 6953-6962.
5. Mallery DL, Vandenberg CJ, Hiom K. Activation of the E3 ligase function of the BRCA1/BARD1 complex by polyubiquitin chains. *EMBO J* 2002;21: 6755-6762.
6. Kittler R, Pelletier L, Heninger AK, Slabicki M, Theis M, Miroslaw L, Poser I, Lawo S, Grabner H, Kozak K, Wagner J, Surendranath V, Richter C, Bowen W, Jackson AL, Habermann B, Hyman AA, Buchholz F. Genome-scale RNAi profiling of cell division in human tissue culture cells. *Nat Cell Biol* 2007;9: 1401-1412.
7. Yu H, Mashtalir N, Daou S, Hammond-Martel I, Ross J, Sui G, Hart GW, Rauscher FJ, 3rd, Drobetsky E, Milot E, Shi Y, Affar el B. The ubiquitin carboxyl hydrolase BAP1 forms a ternary complex with YY1 and HCF-1 and is a critical regulator of gene expression. *Mol Cell Biol* 2010;30: 5071-5085.
8. Machida YJ, Machida Y, Vashisht AA, Wohlschlegel JA, Dutta A. The deubiquitinating enzyme BAP1 regulates cell growth via interaction with HCF-1. *J Biol Chem* 2009;284: 34179-34188.
9. Murali R, Wiesner T, Scolyer RA. Tumours associated with BAP1 mutations. *Pathology* 2013;45: 116-126.

10. Carbone M, Yang H, Pass HI, Krausz T, Testa JR, Gaudino G. BAP1 and cancer. *Nat Rev Cancer* 2013;13: 153-159.
11. Knudson AG, Jr. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A* 1971;68: 820-823.
12. Harbour JW, Onken MD, Roberson ED, Duan S, Cao L, Worley LA, Council ML, Matatall KA, Helms C, Bowcock AM. Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science* 2010;330: 1410-1413.
13. Yoshikawa Y, Sato A, Tsujimura T, Emi M, Morinaga T, Fukuoka K, Yamada S, Murakami A, Kondo N, Matsumoto S, Okumura Y, Tanaka F, Hasegawa S, Nakano T, Hashimoto-Tamaoki T. Frequent inactivation of the BAP1 gene in epithelioid-type malignant mesothelioma. *Cancer Sci* 2012;103: 868-874.
14. Testa JR, Cheung M, Pei J, Below JE, Tan Y, Sementino E, Cox NJ, Dogan AU, Pass HI, Trusa S, Hesdorffer M, Nasu M, Powers A, Rivera Z, Comertpay S, Tanji M, Gaudino G, Yang H, Carbone M. Germline BAP1 mutations predispose to malignant mesothelioma. *Nat Genet* 2011;43:1022-1025
15. Carbone M, Ferris LK, Baumann F, Napolitano A, Lum CA, Flores EG, Gaudino G, Powers A, Bryant-Greenwood P, Krausz T, Hyjek E, Tate R, Friedberg J, Weigel T, Pass HI, Yang H. BAP1 cancer syndrome: malignant mesothelioma, uveal and cutaneous melanoma, and MBAITs. *J Transl Med* 2012;10: 179.
16. Wiesner T, Obenaus AC, Murali R, Fried I, Griewank KG, Ulz P, Windpassinger C, Wackernagel W, Loy S, Wolf I, Viale A, Lash AE, Pirun M, Socci ND, Rutten A, Palmedo G, Abramson D, Offit K, Ott A, Becker JC, Cerroni L, Kutzner H, Bastian BC, Speicher MR. Germline mutations in BAP1 predispose to melanocytic tumors. *Nat Genet* 2011;43: 1018-1021.
17. Abdel-Rahman MH, Pilarski R, Cebulla CM, Massengill JB, Christopher BN, Boru G, Hovland P, Davidorf FH. Germline BAP1 mutation predisposes to uveal melanoma, lung adenocarcinoma, meningioma, and other cancers. *J Med Genet* 2011;48: 856-859.
18. Popova T, Hebert L, Jacquemin V, Gad S, Caux-Moncoutier V, Dubois-d'Enghien C, Richaudeau B, Renaudin X, Sellers J, Nicolas A, Sastre-Garau X, Desjardins L, Gyapay G, Raynal V, Sinilnikova OM, Andrieu N, Manie E, de Pauw A, Gesta P, Bonadona V, Maugard CM, Penet C, Avril MF, Barillot E, Cabaret O, Delattre O, Richard S, Caron O, Benfodda M, Hu HH, Soufir N, Bressac-de Paillerets B, Stoppa-Lyonnet D, Stern MH. Germline BAP1 mutations predispose to renal cell carcinomas. *Am J Hum Genet* 2013;92: 974-980.

19. Rusch VW. A proposed new international TNM staging system for malignant pleural mesothelioma from the International Mesothelioma Interest Group. *Lung Cancer* 1996;14: 1-12.
20. Firmann M, Mayor V, Vidal PM, Bochud M, Pecoud A, Hayoz D, Paccaud F, Preisig M, Song KS, Yuan X, Danoff TM, Stirnadel HA, Waterworth D, Mooser V, Waeber G, Vollenweider P. The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome. *BMC Cardiovasc Disord* 2008;8: 6.
21. Aoude LG, Vajdic CM, Kricker A, Armstrong B, Hayward NK. Prevalence of germline BAP1 mutation in a population-based sample of uveal melanoma cases. *Pigment Cell Melanoma Res* 2013;26: 278-279.
22. Njauw CN, Kim I, Piris A, Gabree M, Taylor M, Lane AM, DeAngelis MM, Gragoudas E, Duncan LM, Tsao H. Germline BAP1 inactivation is preferentially associated with metastatic ocular melanoma and cutaneous-ocular melanoma families. *PLoS One* 2012;7: e35295.
23. Sauna ZE, Kimchi-Sarfaty C. Understanding the contribution of synonymous mutations to human disease. *Nat Rev Genet* 2011;12: 683-691.

8. Legends to the figures

Figure 1.

Electropherogram of DNA alteration in exon 11 of BAP1 gene in patient 76. The synonymous variation c.1026C>T does not lead to a change in the encoded amino acid (p.Ser342=) It is reported on Ensembl and dbSNP database (rs71651686).

Figure 1

